THE INCIDENCE OF FUNGI IN HUMAN AXILLARY HAIR AND THEIR TOXIGENIC POTENTIALITIES

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ABSTRACT The present study determined the incidence of fungi in 66 specimens of human axillary hair Eleven genera, 22 species and 2 varieties were isolated *Penicillium funiculosum* (32% of hair specimens) was the only fungus isolated in moderate incidence *Aspergillus fumigatus* (21%), *A flavus* (17%), *A niger* (17%) and *Chrysosporium tropicum* (12%) showed low incidence However, *Trichosporon catenulatum, Trichophyton mentagrophytes, Candida albicans, Chrysosporium keratinophilum* and *Geotrichum candidum* were rare Twenty-eight isolates (out of 57 tested isolates) belonging to the most common fungal species (*A flavus A fumigatus* and *P funiculosum*) recovered from axillary hair produced respective mycotoxins The detected toxins were aflatoxins B_1, B_2 G_1, G_2, gliotoxin and ochratoxins A, B The selective effects of Bac (sweet deodorant) on the mycelial dry weight, protease activity and mycotoxins production of some fungal species were also examined At 1% concentration Bac inhibited the mycelial dry weight of all tested fungi and protease activity of *A flavus* and *A fumigatus* However, it promoted protease activity of *A niger* and *P funiculosum*. The specific production of aflatoxins by *A flavus* and ochratoxins by *P funiculosum* showed their accumulation with Bac treatment

RÉSUMÉ 22 espèces et 2 variétés de champignons, appartenant à 11 genres, ont été isolées de poils axillaires humains *Penicillium funiculosum* (32% des échantillons de poils) est la seule espèce présentant une fréquence notable d'isolement *Aspergillus fumigatus* (21%), *A flavus* (17%), *A niger* (17%) et *Chrysosporium tropicum* (12%) présentent des fréquences d'isolement faibles *Trichosporon catenulatum, Trichophyton mentagrophytes, Candida albicans Chrysosporium keratinophilum* et *Geotrichum candidum* sont rarement isolés 38 isolements (sur 57 isoles) appartenant aux espèces les plus fréquentes (*A flavus, A fumigatus P funiculosum*) produisent des toxines Les toxines isolées sont les aflatoxines B1, B2, G1 et G2, la gliotoxine et les ochratoxines A et B L'action du "Bac" (déodorant) sur la croissance mycélienne, l'activité protéasique et la production de mycotoxines a été étudiée pour un certain nombre d'espèces A une concentration de 1% le "Bac" inhibe la croissance mycélienne (poids secs) de toutes les espèces étudiées et l'activité protéasique d'*Aspergillus flavus* et d'*Aspergillus fumigatus* Au contraire, à cette même concentration, l'activité protéasique d'*Aspergillus niger* et de *Penicillium funiculosum* est stimulée La concentration mycélienne en aflatoxine et en ochratoxine respectivement chez *Aspergillus flavus* et *Penicillium funicusolum* est augmentée par le traitement au "bac"

KEY WORDS fungi, human axillary hair, mycotoxins, protease activity sweat deodorant

INTRODUCTION

The isolation of dermatophytes and other non dermatophytes from hair has been investigated by several authors (English, 1976, Takotori & Ichijo, 1979, Lopez

Martinez & Rivera Lona, 1984 and Imwidthaya & Thianprasite, 1988) Dermatophytes utilize protein as the main nutrient source and are capable of decomposing even a resistant scleroprotein, such as keratin During their growth on protein substrates they secrete strongly active proteolytic enzymes Mycotoxins are toxic substances produced by fungi which cause diseases in animals or man Acute diseases caused by mycotoxins are called mycotoxicoses No available literatures concerning with the ability of fungi isolated from human axillary hair for producing mycotoxins Therefore, the present investigation deals with the incidence of fungi in human axillary hair and their toxigenic potentialities to establish whether a potential hazard might exist due to contamination of hair with toxigenic molds Also, the selective effects of one of sweat deodorants on the mycelial growth, protease activity and mycotoxin production by some fungal species were examined

MATERIALS AND METHODS

Isolation and identification of fungi:

Sixty-six specimens of human axillary hair were examined These specimens were randomly chosen from healthy males in Assiut city The specimens were placed in sterilized Petri-dishes and sent immediately to the laboratory A portion of specimens were examined in 10% KOH for the presence of fungal hyphae and arthrospores The specimens were also cultured on Sabouraud's dextrose agar containing 0 05 mg/ml of chloramphenicol and 0 5 mg/ml cycloheximide The plates were incubated at 28°C (± 2) and the fungal growth was observed once weekly for a period of 4 weeks The developing colonies were examined microscopically and identified

Toxigenic potentialities of common fungi:

Fifty-seven isolates belonging to *Aspergilus flavus*, *A fumigatus*, *A niger* and *Penicillium funiculosum* which represent the most common fungal species associated with hair were subjected to mycotoxin screening during this investigation Twenty ml of YES (yeast extract sucrose) medium in 100 ml Erlenmeyer flask were sterilized, inoculated with 1 ml spore suspension of 1 week old culture of each tested isolate and incubated at 25°C (+ 2) for 7 days as stationary cultivation Extraction and analysis of the fungal toxins are carried out as mentioned below

Antimycotic activity of sweat deodorant:

One type of sweat deodorant (Bac) was tested for its antimycotic activity Forty ml of sterilized Sabouraud's dextrose medium were poured into 250 ml Erlenmeyer flask Bac (manufactured by UTAC, Egypt under licence from Hans Schwarzkopf GmbH Hamburg, Germany) was incorporated at concentrations 0 5, 1 0 and 5 0% Two flasks were used for each concentration and control (free from Bac) Then, the flasks were inoculated with 1 ml spore suspension (approx 10^6 spores) and incubated as described previously

Estimation of protease activity:

Proteolytic enzyme activity in media was measured using casein powder as substrate The reaction mixture containing 2 ml of 1% (w/v) casein in 0 1 M sodium phosphate buffer pH 7 6 was incubated with 1ml of culture filtrate at 30°C (+ 2) for 2 h The reaction was stopped and unhydrolyzed protein was removed by addition of

1ml of 50% trichloroacetic acid The precipitated undigested protein was removed by centrifugation The hydrolyzed protein in supernatant was determined by the method of Lowry et al. (1951).

Extraction and analysis of the fungal toxins:

The content of each flask (mycelium + filtrate) was homogenized for 5 min in high speed blender with 50 ml chloroform Then the extract was evaporated till dryness on a rotary evaporator The chloroform extracts were analysed for the presence of mycotoxins on Silica Gel coated plates The methods of Nesheim (1976), Moss & Badii (1982) and Richard et al (1989) were used for analyses of of ochratoxin, aflatoxin and gliotoxin, respectively Quantitative estimation of these toxins was made according to Nabney & Nesbitt (1965) and Applegate & Chipley (1976)

RESULTS AND DISCUSSION

A total of 11 genera, 22 fungal species and 2 varieties were isolated from healthy human axillary hair collected from 66 males in Assiut city *Penicillium funiculosum* was moderately isolated, being present in 32% of the specimens Four fungal species showd low incidence viz *Aspergillus fumigatus, A flavus, A niger* and *Chrysosporium tropicum* However, the remaining fungal species were rare in human axillary hair (Table 1)

Dermatophytes were represented by five species belonging to *Chrysosporium* (2 species), *Trichosporon, Trichophyton* and *Candida* (1 species for each) According to the percentage inncidence (in relation to the total specimens) these species could be arranged in the follwing order *C tropicum* (12%) > *Trichosporon catenulatum* (6) > *Trichophyton mentagrophytes* (4.5) > *C keratinophilum* and *Candida albicans* (1.5 for each) Moharram et al (1988) noticed that *C tropicum, C keratinophilum, C lobatum* and *C queenslandicum* were associated with healthy human hair In Iran, Mognadami & Emami (1986) reported that dermatophytes isolated from tinea capitis included *Microsporum canis, Trichophyton violaceum, T verrucosum, T. schoenleinii* and 1 isolate of each of *T. mentagrophytes, Candida albicans* and *Candida* sp

Seventeen species and 2 varieties belonging to 7 genera of fungi other than dermatophytes were associated with axillary hair *Penicillium funiculosum, Aspergillus fumigatus, A flavus* and *A niger* were the dominant molds recovered (32, 21, 17 and 17% of the specimens, respectively).

Yeast (red colour), *P. chrysogenum, A. flavus* var. *columnaris, Alternaria alternata, Cladosporium cladosporioides, Emericella nidulans* var *dentata* and *Mucor racemosus* were associated with 3-11% of the examined hair El-Shanawani (1993) reported that several saprophytic fungi were recovered from tinea capitis in Assiut and New Valley Governorates, the most common species were *A flavus, A niger, A fumigatus, P chrysogenum, Alternaria alternata* and *Cladosporium cladosporioides* Also, El-Gendy (1988) and Mahmoud (1991) isolated *A flavus, A niger, A sydowii, P chrysogenum, Alt alternata* and *C cladosporioides* from cases of tinea capitis English (1965) observed that *A fumigatus* and *A terreus* were able to grow on human hair Whereas, Botticher (1966) recorded that *Alternaria alternata* has been implicated as a cause of dermitis in human In this study, each of *A carneus, A terreus A ustus, Cladosporium carrionii, C herbarum, Geotrichum candidum, P canescens, P cyclopium* and *P purpurogenum* are encountered in 1.5% of human axillary hair (Table 1) In this respect, Aho (1983) suggested that the presence of saprophytic fungi on

Genera and species	NCI	Incidence %	OR
Alternaria alternata (Fries) Keissler	2	3.0	R
Aspergillus carneus (V.Tiegh.)Blochwitz	1	1.5	R
A. flavus Link	11	17.0	L
A. flavus var. *columnaris* Raper & Fennell	3	4.5	R
A. fumigatus Fresenius	14	21.0	L
A. niger Van Tieghem	11	17.0	L
A. terreus Thom	1	1.5	R
A. ustus (Bain.) Thom & Church	1	1.5	R
Candida albicans (Robin) Berkhout	1	1.5	R
Chrysosporium keratinophilum (Frey) Carmichael	1	1.5	R
C. tropicum Carmichael	8	12.0	L
Cladosporium carrionii Trejos	1	1.5	R
C. cladosporioides (Fres.) de Vries	2	3.0	R
C. herbarum (Pers.) Link ex Fr.	1	1.5	R
Emericella nidulans var. *dentata* Sandhu & Sandhu	2	3.0	R
Geotrichum candidum Link	1	1.5	R
Mucor racemosus Fresenius	2	3.0	R
Penicillium canescens Sopp	1	1.5	R
P. chrysogenum Thom	6	9.0	R
P. cyclopium Westling	1	1.5	R
P. funiculosum Thom	21	32.0	M
P. purpurogenum Stoll	1	1.5	R
Trichophyton mentagrophytes (Robin) Blanchard	3	4.5	R
Trichosphoron catenulatum (De Berum, Gougerot & Jauchel) Ota	4	6.0	R
Yeast (red colour)	7	11.0	R
Total number of genera	11		
Total number of species and varieties	22+2		

NCI = Number of cases of isolation (out of 66 specimens); OR=Occurrence remarks M=Moderate occurrence (between 17-32 cases) L Low occurrence (between 8-16 cases); R=Rare occurrence (between 1-7 cases).

Table 1 Incidence of fungi in human axillary hair on Sabouraud's dextrose agar at 28°C

Tableau 1 fréquence des espèces fongiques isolées de poils axillaires humains sur milieu de Sabouraud à 28°C

hair and skin creates an opportunity for them under special circumstances to become invasive to the skin or hair and thus cause primary or secundary infection

The results in table (2) show the toxigenic potentialities of fifty-seven isolates belonging to the most common species. 61% of *Aspergillus* members tested produced at least one toxin. All tested isolates of *A. flavus* produced aflatoxins from which two isolates produced aflatoxins B_1, B_2, G_1 and G_2. The remaining 9 isolates produced B_1 and B_2 only. These results are in accordance with the finding of Joffe (1969) who reported that 8.4% of *A. flavus* members produced all four aflatoxins. However, Youssef (1986) reported that 29 isolates of *A. flavus* (out of 30 tested isolates) produced all four aflatoxins, and one isolate formed aflatoxins B_1 and B_2 only. Aflatoxins are mutagenic, carcinogenic, teratogenic and acutely toxic to most experimental and domesticated animals and man (El-Zawahri et al., 1977, Davis & Diener, 1978).

Species	No.of positive specimens	No.of toxin producers	Mycotoxin produced
A flavus	11	9	Aflatoxin B_1, B_2
		2	Aflatoxin B_1, B_2 j, $_2$
A. fumigatus	14	11	Gliotoxin
A. niger	11	0	-ve
P. funiculosum	21	6	Ochratoxin A ,b
Total isolates	57	28	

Table 2 Mycotoxin production by common fungal species associated with axillary hair

Tableau 2 Production de mycotoxine par les espèces fongiques associées aux poils axillaires humains

Production of gliotoxin by 11 isolated of *A fumigatus* out of fourteen tested isolates agree with the finding of Moss (1977) and Richard et al (1989). *A fumigatus* represents the predominant pathogen of aspergillosis in man Eichner & Muilbacher (1984) have hypothesized that gliotoxin may be produced during the pathogenic state of *A fumigatus* and contributes to the pathogenicity of the fungus. Six isolates (out of 21) of *P funiculosum* produced ochratoxins A and B. Carlton & Krogh (1979) classified *P funiculosum* as ochratoxins producer. Ochratoxin A causes nephropathy in pigs (Elling et al 1985) and induces renal and hepatic carcinomas in mice (Kanisawa & Suzuki, 1978, Bendele et al 1983) Data obtained in this investigation, proved that most of fungi tested produced seven mycotoxins These data strengthen our initial concern that potential hazard to human health may exist due to the presence of toxigenic fungi on hair

The selective effect of sweat deodorant (Bac) on mycelial growth and protease activity of 5 isolates of fungal species associated with hair was illustrated in table (3) The mycelial growth of three species species of *Aspergillus* namely *A flavus, A fumigatus* and *A niger* were inhibited at 1% concentration whereas the mycelial dry weight of *Penicillium funiculosum* and *Chrysosporium tropicum* were more sensitive at 0 5% concentration The main constituents of Bac are water, SD alcohol 39 C, aluminium chlorhydrate, ceteareth 11, Fragrance and hydroxy ethylcellulose According to Megalla et al (1980), the essential oils as aliphatic alcohols exhibited a high antifungal activity Moharram et al (1988, used creams, vaseline, shampoos and oils which applied to human hair for treatment of dry skin and dandruff, as antimycotic agent They found that silk hair with lanolin cream (yellow) Relax bath (blue) and Silk hair (herb-green) were highly effective against all tested fungi Also, *Chrysosporium* isolates were the most sensitive fungi to shampoos

The ability of five fungal species to produce extracellular protease in broth cultures was found to be greatly affected by the addition of sweat deodorant (Bac) as shown in table (3) The response seemed to be fluctuated between inhibition and promotion Protease synthesis by *A flavus* and *A fumigatus* was greatly reduced at

Species	Conc. %	Mycelial dry wt (mg/40 ml)	Sporulation	Protease activity (µg/ h/ml)
A. flavus	0	282	+	766.3
	0.5	264	+	630.0
	1.0	181	+	462.5
	5.0	no growth		
A.fumigatus	0	235	+	283.8
	0.5	223	+	230.0
	1.0	198	−	202.5
	5.0	no growth		
A. niger	0	394	+	126.3
	0.5	349	+	200.0
	1.0	125		165.0
	5.0	no growth		
Chrysosporium tropicum	0	249	+	223.8
	0.5	122	+	232.5
	1.0	no growth		
P. funiculosum	0	310	+	153.8
	0.5	196	+	205.0
	1.0	162	−	212.5
	5.0	no growth		

Table 3 Effect of sweat deodorant (Bac) on mycelial growth, sporulation and protease activity of five species.

Tableau 3 Effets d'un déodorant (Bac) sur la croissance mycélienne la sporulation et l'activité protéasique de 5 espèces fongiques

concentration 1% In this respect, Mahmoud (1991) found that citral, citronellol, nerol and caproic acid reduced protease activity in *Fusarium compactum* and *Trichophyton violaceum*

Concentration of 0.5% of Bac has promotive effect on protease activity in *A niger, P funiculosum* and *C tropicum* Carvone and Thymol were found to be accelerate protease production by *F compactum* and *T violaceum* (Mahmoud, 1991 Davidson & Branen (1980, found that the antioxidant such as butylated hydroxy anisole and butylated hydroxy toluene caused leakage of intracellular protein from *Pseudomonas fluorescens* Cumming (1990) concluded that aluminium has inhibition of nitrate reductase activity in roots of pitch pine (*Pinus rigida*) seedlings

Conc %	*A flavus* Mycelial dry wt (mg/40 ml)	Aflatoxin (µg/flask) B_1+B_2	Aflatoxin (µg/flask) G_1+G_2	Aflatoxin (µg/flask) Total	Specific production (µg/g dry wt) B_1+B_2	Specific production (µg/g dry wt) G_1+G_2	Specific production (µg/g dry wt) Total	*P. funiculosum* Mycelial dry wt (mg/40 m.)	Ochratoxin (µg/flask) A	Ochratoxin (µg/flask) B	Ochratoxin (µg/flask) Total	Specific production (µg/g dry wt) A	Specific production (µg/g dry wt) B	Specific production (µg/g dry wt) Total
0	282	60 3	38 4	98 7	213 8	136 2	350 0	3.0	88.0	52.0	140	283.9	167 7	451.6
0 5	264	61 2	37 8	99 0	231.8	143 2	375 0	196	105.0	33.0	138	535.7	168.4	704.1
1 0	181	62.5	36.1	98.6	345 3	199 4	544.7	162	120.0	21.0	141	740.7	129 6	770.3
5 0	no growth					no growth								

Table 4 Effect of sweat deodorant (Bac) on aflatoxins production by *A flavus* and ochratoxins production by *P funiculosum* in Sabouraud's dextrose liquid medium

Tableau 4 effets d'un deodorant (Bac) sur la production d'aflatoxines par *A flavus* et d'ochratoxines par *P funiculosum* sur le milieu liquide de Sabouraud

The mechanism of fungal inhibition might be due to the Bac constituents act on the cytoplasmic membrane and might also be related to the destruction or inactivation of essential enzymes and/or genetic material These reasons are agreed with the conclusion of Branan et al (1980) on the mechanism of antioxidants on microbial inhibition.

The selective effect of sweat deodorant (Bac) on the secondary metabolites (aflatoxins and ochratoxins) of two toxigenic molds isolated from human axillary hair (*A flavus* and *P funiculosum*) was illustrated in table (4) The inhibitory action of Bac on mycelial growth of *A flavus* has no effect on aflatoxin production However, the low specific production shows highly accumulation of toxins with treatments In this respect, Hasan & Mahmoud (1993) reported that the specific production of aflatoxins increased at concentration 250 ppm of cumin and 500 ppm of clove oils

The results of action of Bac on ochratoxins production by *P. funiculosum* (Table 4) show that the active constituents of Bac maintained the ochratoxin B inhibitory properties possibly by interfering with its biosynthesis pathway On the other hand, ochratoxin A was accumulated at 0 5 and 1% concentrations Bac may be incorporated into precursors of ochratoxins instead of the correct component in the biosynthetic pathway and thus inhibit toxin B formation

Our results indicate that, Bac has the unique capability of selectively altering ochratoxin synthesis in *P funiculosum* strain which produced both A and B type toxins Large increases in the amounts of ochratoxin A with corresponding decreases in B toxin may be due to specific inhibition of the conversion of A to B by Bac

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